GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: 10/17/80

Project Title: Non-Heme Iron Oxygenase Catalysis

Project No: G-33-H04

Project Director: Dr. Sheldon W. May

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences

Agreement Period: From 9/1/80 Until 8/31/81 (04 year)

Type Agreement: Grant No. 2 R01 GM23474-04

Amount: $ 98,568 New PHS Funds (G-33-H04)
5,523 GIT Contribution (G-33-327)
$104,091 Total

Reports Required: Annual Progress Reports with Continuation Applications
Terminal Progress Report upon Grant Expiration

Sponsor Contact Person (s):

Technical Matters

Program Administrator: Dr. Marvin Cassman
301/496-7463
Program Official: Arthur E. Heming, PhD.
Associate Director for Program Activities
National Institute of General Medical Sciences
Bethesda, MD 20014

Contractual Matters
(thru OCA)

Evelyn W. Carlin
Grants Management Officer
Office of Assoc. Director for Progra
Activities
National Institute of General Medica
Sciences
Bethesda, MD 20014

Ruth C. Monaghan /Linda V. Glen
Grants Management Specialist
301/496-7746

NOTE: FOLLOW-ON PROJECT TO G-33-H03

Defense Priority Rating: None

Assigned to: Chemistry (School/Laboratory)

COPIES TO:

Project Director
Division Chief (EES)
School/Laboratory Director
Dean/Director–EES
Accounting Office
Procurement Office
Security Coordinator (OCA)
Reports Coordinator (OCA)
Property Coordinator (OCA)

Library, Technical Reports Section
EES Information Office
EES Reports & Procedures
Project File (OCA)
Project Code (GTRI)
Other

CA-3 (3/76)

B-440

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

Date: August 20, 1981

Project Title: Non-Heme Iron Oxygenase Catalysis

Project No: G-33-H04

Project Director: Dr. Sheldon M. May

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences

Effective Termination Date: 8/31/81

Clearance of Accounting Charges: -----

Grant/Contract Closeout Actions Remaining:

_ Final Invoice and Closing Documents
_ Final Fiscal Report
X Final Report of Inventions
_ Govt. Property Inventory & Related Certificate
_ Classified Material Certificate
X Other Annual Report of Expenditures due by 11/30/81

NOTE: Follow-on project (05 year) is G-33-H05

Assigned to: Chemistry (School/[redacted])

COPIES TO:

Administrative Coordinator
Research Property Management
Accounting Office
Procurement Office
Research Security Services
~~Reports Coordinator (OCA)~~

Legal Services (OCA)
Library, Technical Reports
EES Research Public Relations (2)
Project File (OCA)
Other: _____

G 33-H04

GEORGIA INSTITUTE OF TECHNOLOGY
ATLANTA, GEORGIA 30332

OFFICE OF
THE
COMPTROLLER

January 13, 1981

Grants Management Officer
DHHS/PHS/NIH
Office of Associate Director
for Program Activities
National Institute of General
Medical Sciences
Bethesda, MD 20205

Dear Sir or Madam:

Enclosed is the Annual Report of Research Grant Expenditures for Grant No.5 R01 GM23474-03 for the period 9/1/79 - 8/31/80.

If you have questions or require additional information, please let us know.

Sincerely,

David V. Welch

David V. Welch, Manager
Grants and Contracts Accounting

DVW/BITS/jb
Enclosure
cc: Dr. S. W. May
Dr. J. A. Bertrand
Mr. J. W. Dees
Mr. O. H. Rodgers
File G-33-H03

DEPARTMENT OF HEALTH AND HUMAN SERVICES

(Instructions are on reverse)

NAME AND ADDRESS OF GRANTEE INSTITUTION	TRANSACTION NO.	Grant No.
Georgia Institute of Technology Atlanta, Georgia 30332	(08)R1GM23474 A	5 R01 GM23474-03
	INSTITUTIONAL ID NO.	DATE OF THIS REPORTING PERIOD
	G-33-H03	FROM 9/1/79 TO 8/31/80
		PROJECT PERIOD
		FROM 9/1/77 TO 8/31/83
		☐ CHECK IF FINAL REPORT

1. Expenditures of DHHS Funds for this Reporting Period

a. Personnel	$	h. Alterations and renovations	
b. Consultant services		i. Other	
c. Equipment			
d. Supplies		j. Total direct costs	41,246.00
e. Travel, domestic		k. Indirect costs: Rate * % ☒ S&W ☐ TDC	
f. Travel, foreign		Base $ *	20,520.00
g. Patient care costs		l. TOTAL	$ 61,766.00

2. Expenditures from Prior Periods (previously reported)	109,661.77
3. Cumulative Expenditures	171,427.77
4. Total Amount Awarded – Cumulatively	171,820.00
5. Unexpended Balance (Item 4 less Item 3)	392.23
6. Unliquidated Obligations	-
7. Unobligated Balance (Item 5 less Item 6)	392.23
8.a. Cost Sharing Information – Grantee Contribution This Period	3,730.20
b. % of Total Project Costs (Item 8a divided by total of Items 1 and 8a)	% 5.7
9.a. Interest/Income (enclose check)	-
b. Other Refundable Income (enclose check)	-

10. Remarks * 9/1/79 to 6/30/80 — 76% X $ 20,346.83 = $ 15,463.59
As of 7/1/80 — 73% X 8,619.48 = 6,292.22
$ 28,966.31 $ 21,755.81

To be reported on SROEAS

I hereby certify that this report is true and correct to the best of my knowledge, and that all expenditures reported herein have been made in accordance with appropriate grant policies and for the purposes set forth in the application and award documents.

Dr. S. W. May — Assoc. Professor — Date 1/9/81

David V. Welch

SIGNATURE OF INSTITUTION OFFICER — DATE

David V. Welch, Manager, Grants & Contracts Acctg.
404/894-4624

Formerly HEW-489

REPORT OF RESEARCH GRANT EXPENDITURES

SERVES AS INTERIM PROGRESS REPORT

SECTION I

OMB No. 68-R0249

DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

APPLICATION FOR CONTINUATION GRANT

REVIEW GROUP	TYPE	PROGRAM	GRANT NUMBER (INSERT ON ALL PAGES)
PB	5	R01	GM23474-05

TOTAL PROJECT PERIOD
FROM: 09/01/77 THROUGH: 08/31/83

REQUESTED BUDGET PERIOD
FROM: 09/01/81 THROUGH: 08/31/82

TO BE VERIFIED BY APPLICANT. CHECK INFORMATION IN ITEMS 1 THROUGH 6. IF INCORRECT, FURNISH CORRECT INFORMATION IN ITEM 13.

1. TITLE

NON-HEME IRON OXYGENASE CATALYSIS

2A. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Name and Address, Street, City, State, Zip Code)

MAY, SHELDON W
GEORGIA INST OF TECHNOLOGY
SCHOOL OF CHEMISTRY
ATLANTA, GA 30332

4. APPLICANT ORGANIZATION (Name and Address, Street, City, State, Zip Code)

GEORGIA INSTITUTE OF TECHNOLOGY
225 NORTH AVENUE, N W
ATLANTA, GA 30332

2B. DEGREE: PHD

2C. SOCIAL SECURITY NO.: 320-44-5583

5. PHS ACCOUNT NUMBER: 1586002023A1

2D. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT

SCHOOL OF CHEMISTRY

2E. MAJOR SUBDIVISION

COLL OF SCIS & LIBERAL STUDIES

3. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR INSTITUTIONAL GRANT PURPOSES

20 OTHER

6. TITLE AND ADDRESS OF OFFICIAL IN BUSINESS OFFICE OF APPLICANT ORGANIZATION

COMPTROLLER
GEORGIA INSTITUTE OF TECHNOLOGY
ATLANTA, GA 30332

COMPLETE THE FOLLOWING (See Instructions)

7. RESEARCH INVOLVING HUMAN SUBJECTS (See Instructions)
☒ NO ☐ YES
APPROVED: _____ DATE

8. INVENTION CERTIFICATION (See Instructions)
☐ NO ☐ YES-NOT PREVIOUSLY REPORTED ☐ YES-PREVIOUSLY REPORTED

9. PERFORMANCE SITE (S)

Georgia Institute of Technology
School of Chemistry
Atlanta, Georgia 30332

TELEPHONE INFORMATION

	AREA CODE	TELE. NO. & EXT.
11A. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (ITEM 2A)	404	894-4052
11B. NAME OF BUSINESS OFFICIAL (ITEM 6) Frank Huff Comptroller	404	894-4622
11C. NAME AND TITLE OF ADMINISTRATIVE OFFICIAL (ITEM 15B) Jerry Goldbaugh Contracting Officer	404	894-4814

10. DIRECT COSTS REQUESTED FOR BUDGET PERIOD: $77,737

12A. CONGRESSIONAL DISTRICT OF APPLICANT ORGANIZATION SHOWN IN ITEM 4: 5th Congressional District

12B. COUNTY OF APPLICANT ORGANIZATION SHOWN IN ITEM 4: Fulton

13. USE THIS SPACE FOR CORRECTIONS TO ITEMS 1 THROUGH 6. INDICATE THE NUMBER(S) WHERE ANSWER(S) APPLY

13A IS RECOM. DNA RESEARCH SUBJECT TO NIH GUIDELINES INVOLVED? NO( ) YES( )
(IF YES, AN UPDATED MUA IS REQUIRED)

14. CERTIFICATION AND ACCEPTANCE. WE, THE UNDERSIGNED, CERTIFY THAT THE STATEMENTS HEREIN ARE TRUE AND COMPLETE TO THE BEST OF OUR KNOWLEDGE AND ACCEPT, AS TO ANY GRANT AWARDED, THE OBLIGATION TO COMPLY WITH PUBLIC HEALTH SERVICE TERMS AND CONDITIONS IN EFFECT AT THE TIME OF THE AWARD.

SIGNATURES (Signatures required on original copy only. Use ink. "Per" signatures not acceptable.)		DATE
	15A. PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR	6/12/81
	15B. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION: Jerry Goldbaugh / DWIGHT L. ALLEN, DEPUTY DIRECTOR, OFFICE OF CONTRACT ADMINISTRATION	6/15/81

PHS 2590 OPTIONAL REV 4-75

RETURN COMPLETED APPLICATION TO PHS AS SOON AS POSSIBLE:
NO LATER THAN 1 JULY 1981

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE	GRANT NUMBER	
SECTION IV—SUMMARY PROGRESS REPORT	2 RO1 GM 23474-05	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)	PERIOD COVERED BY THIS REPORT	
MAY, Sheldon W.	FROM	THROUGH
NAME OF ORGANIZATION Georgia Institute of Technology/School of Chemistry	9/1/81	8/31/82

TITLE (Repeat title shown in Item 1 on first page)
Non-Heme Iron Oxygenase Catalysis

1. List all publications, not previously reported, resulting from work supported by this grant (author(s), title, page numbers, year, journal or book). List manuscripts separately as submitted for publication or accepted for publication
2. Provide two reprints of publications not previously submitted to the awarding unit.
3. Progress Report. (See instructions)

1. R.S. Phillips and S.W. May, "Enzymatic Sulfur Oxygenation Reactions", Enz. Microb. Technol., 3, 9-18 (1981).

S.W. May, R.S. Phillips, P.W. Mueller and H.H. Herman, "Dopamine-β-Hydroxylase: Demonstration of Enzymatic Ketonization of the Product Enantiomer S-octopamine", J. Biological Chem., 256, 2258-2261 (1981).

S.W. May, R.S. Phillips, P.W. Mueller and H.H. Herman, "Dopamine-β-Hydroxylase: Comparative Specificities and Mechanisms of the Oxygenation Reaction" J. Biological Chem., (In Press).

S.W. May, S. Goel, A.L. Sowell, L. Barrow and R.H. Felton, "Protocatechuate-3,4-Dioxygenase: Raman Studies on the ^{54}Fe and ^{34}S Isotopically Substituted Species", In Preparation.

3. Progress Report

Non-heme iron-containing oxygenases represent more than 80% of all know dioxygenases and a large number of mono-oxygenases, and thus, a definition of the molecular basis of their catalytic action is highly relevant to many key biological processes. The broad objectives of our research program are to continue our analysis of the involvement of non-heme iron in the catalytic pathway of bacterial dioxygenases, and to initiate comparative studies with mamalian mono-oxygenases containing functional non-heme iron or copper. In these studies we utilize physical techniques such as resonance Raman and EXAFS chemical tecnhiques such as the design of transition state analogs and other active-site directed ligands, metal replacement and chemical modification; and rapid reaction kinetic techniques.

The following paragraphs summarize our progress during the first year of this project.

^{34}S-Protocatechuate-3,4-dioxygenase (PCD): In previous studies of the resonance Raman spectra of PCD the presence of a band at 274 cm^{-1} which disappears with substrate binding suggested to us the possible ligation of cysteine to iron in the active site. To test this possibility we decided to make ^{34}S labeled PCD which sould show a shift in the 274 cm^{-1} band, if it indeed reflects sulfur ligation.

To incorporate the labeled sulfur in the enzyme, cells were grown on a ^{34}S enriched medium developed specifically for this purpose in our laboratory. PCD was then isolated by our standard procedure to give an excellent yield of 200mg of ^{34}S labeled PCD, with a specific activity of 70 U/mg. Resonance Raman spectroscopy of ^{34}S-PCD showed no isotope shifts from the spectrum of the native enzyme, thus providing strong support for the conclusion that the low frequency bands do not arise from sulfur ligation of the

active site iron.

^{54}Fe-PCD. In experiments complimentary to those described above, PCD in which the active site irons were isotopically labeled was prepared by reconstitution of the apoenzyme with ^{54}Fe. We expected that Raman bands arising from iron ligands would exhibit the expected isotope shifts, thus providing further insight into the possible origins of low frequency bands in the 270 cm^{-1} region. ApoPCD with a specific activity of 0.51 U/mg was prepared from native PCD with a specific activity of 56 U/mg. Ferric oxide with 97.6% ^{34}Fe was obtained from Oak Ridge National Laboratories and was reduced to metallic ^{54}Fe. The metallic ^{54}Fe was dissolved in slightly more than a two-fold excess of H_2SO_4 and base was added to just neutralize the pH. The solution was air dried and the $^{54}FeSO_4(NH_4)_2 \cdot 6H_2O$ crystals were dried with acetone and used to reconstitute ApoPCD. The ^{54}Fe labeled PCD had a specific activity of 49.7 U/mg. Resonance Raman spectra of the ^{54}Fe labeled PCD showed that the band found at 274 cm^{-1} in nativePCD had shifted to 277 cm^{-1}. This suggests that the bands at 274 cm^{-1} in native PCD reflects an iron ligand bond, but the identity of the ligand is yet to be established.

EXAFS Studies. In collaboration with Drs. Stern and Parson of the University of Washington, we have now successfully initiated EXAFS Studies with PCD, as set out in our proposal. A concern of the Study Section was that X-ray exposure might denature the protein but our owrk to date with PCD has clearly established that this will not be a problem. PCD samples were sent to the University of Wasington and EXAFS run at the Stanford accelerator, after which the enzyme was returned to us for assay and spectral examination. No significant changes in either activity or spectral properties resulted from X-ray irradiation or shipment of the solutions. Although analysis of the data is not yet complete, the very exciting possibility has arisen that we may have obtained the first evidence for a histidine ligand of the active-site iron of PCD. We are very much encouraged by our success in these initial experiments, and our collaborators at the Univeristy of Washington have indicated that the data obtained are of high quality. Thus, during the coming year we anticipate being heavily involved in obtaining and analyzing EXAFS data both with PCD and other mono-oxygenases, such as Phenylalanine Hydroxylase and, possibly, Dopamine-β-Hydroxylase.

Transition-State Analogs. Our proposed studies with 2-hydroxypyridine-N-oxides as transition state analogs for PCD have produced very promising reuslts. To date, we have successfully completed the very difficult syntheses involved, fully characterized the compounds, and established that our N-oxides are highly potent inhibitors for PCD. Stopped-flow kinetic experiments are now underway in order to characterize the binding events and differentiate ground state from transition state inhibition. It is our expectation that our results will provide strong support for the mechanistic proposals which we have previously made for non-heme iron dixygenase catalysis.

Studies With Other Mono-oxygenases. In experiments which we did not forsee at the start of our program, we have begun to obtain comparative specificity data regarding oxygenase catalysis which we anticipate will allow us to suggest a unified mechanism for enzymatic oxygenation reactions. Working with Dopamine-β-Hydroxylase we have established two new activities for this enzyme -- stereospecific sulfoxidation of sulfides and ketonization of enantiomers of the normal hydroxylation product. We have now successfully carried out a systematic study of both the effects of substrate secondary structure and of various sutstituents on the sulfoxidation and hydroxylation reactions. Our results have allowed us to make direct mechanistic comparisons between Dopamine-β-Hydroxylase, Cytochrome-P-450 and chemical model systems, and we anticipate extending similar studies to Phenylalanine Hydroxylase in the near future. The specific details of our mechanistic suggestions will not be presented in this brief report, but are fully discussed in a paper which is in press in the Journal of Biological Chemistry. Copies of reprints will, ofcourse, be provided when available.

During the coming year, our objectives are to continue and amplify our EXAFS studies with both PCD and mono-oxygenase enzymes, to complete the characterization of our transition state analogs for PCD, and to continue our mechanistic studies aimed at providing unified mechanistic information about the pathway of enzymatic oxygenation reactions. In our view, enough of the preliminary work has now been successfully completed so that we can confidently expect to meet these objectives during the coming year. As always, we are grateful to the National Institutes of Health for continued support of our research efforts.